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A surgical method for implanting transmitters with sensors into the body cavity of cod (*Gadus morhua* L.)

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Abstract

Relatively complicated surgery is a necessity when implanting sensors internally in fish in order to measure physiological variables at specific locations.

A surgical procedure for implanting transmitters with remote sensors into the body cavity of cod (*Gadus morhua* L.) is presented. The method employed introduces new procedures in fish surgery which enable the experimentalist to perform prolonged operations on marine fish.

Introduction

Interest has focused for the last decade on fish telemetry (Stasko & Pincock 1977). Most ultrasonic tags have been used for tracking fish (e.g. Ziebell 1973) but some efforts have been made to direct attention to the measurement of physiological data from fish. Especially, the measurement of the heart beat rate (SINTEF 1977), opercular movements (Rogers & Weatherley 1983) and swimming activity of fish (Weatherley *et al.* 1982) have been studied.

Ultrasonic tags used for tracking fish have most often been attached to the external surface of the animals (Rogers & Weatherley 1983, Henderson *et al.* 1966). Some tags and most heartbeat transmitters have been placed in the stomach (Henderson *et al.* 1966, SINTEF 1975). A problem inherent in these types of attachment is, that external tags may cause a drag and thus alter swimming movements, and stomach tags are frequently lost due to regurgitation (Stasko & Pincock 1977).

The present study describes a method for surgical implantation of dummy transmitters into the abdominal cavity of cod. Implantation was considered advantageous not only because the equipment cannot be lost, but also because the aim of our future studies is the continuous measurement of physiological variables inside the fish.

An ultrasonic transmitter especially designed for abdominal implantation is being developed by the Danish Institute for Fisheries and Marine Research. The

transmitter proper will approximate the form and dimensions of the dummy transmitter.

Surgical implantation of ultrasonic tags have been employed by only few authors. The method described in this study introduces new procedures in fish surgery, which allow the biologist to perform the more complicated surgery involved in the measurement of internal data from fish.

Materials

The anaesthetic was MS222 (Sandoz) dissolved in seawater (1:20000). The suture thread for implantation of the dummy transmitter and dummy sensor was Mersilene 2-0 (Ethicon) or TICRON1 polyesther fiber (Davis & Geck). For suturing the abdominal wall, Mersilene 2-0 (Ethicon), TICRON1 (Davis & Geck), Plain Catgut 2-0 (Ethicon) or Chrom Catgut 2-0 (Ethicon) was used. The surgical implements were pointed, half circle, stainless steel needles (ESH-3, Ethicon) wielded by a needleholder. The dummy transmitters were made of Scotchcast Brand Resin 5 Parts A and B, a material which is used for making pacemakers. The dummy sensors were wires made of silicone rubber. The dimensions of the dummy transmitter were: length 2.5 cm, width 1.9 cm, height 0.75 cm.

The cod (41-48 cm) were caught by trawl, by hook or by pound net. The fish were acclimatized to laboratory conditions in a 3 m^3 tank supplied with running recirculated seawater with a salinity of 30‰ and a temperature within the range of 9.5-14°C.

To monitor the wound postoperatively, a mirror mounted on a rod was used.

Method

Prior to the operation, the fish were starved for 3-4 days. The anaesthesia and operation were performed in a refrigerated room at 10°C. The cod were anaesthetized for 5-10 minutes in a tank holding the MS222 solution. When swimming movements had ceased, and the fish did not react when touched on the caudal peduncle, the cod was transferred to an operating table (Fig. 1). The fish was placed with the ventral surface upwards on a layer of cotton wool moistened by sterile 0.9% salt water. The gill operculae were slightly abducted by a pair of wound retractors, and the gills were continuously irrigated with the anaesthetic by means of a pair of soft PVC tubes connected to a 5 liter tank holding MS222. When the anaesthetic had passed the gills, it was automatically drained from the operating table. An incision was made along the linea alba stretching from 2 cm anterior to the anus in a cranial direction for 2-5 cm. In case of implantation (Table 2), the dummy transmitter and dummy sensor were sutured to the musculature and internal organ respectively by double surgical knots. When suturing the dummy transmitter, allowance was made for movements in the vertical plane, so that the dummy could lodge itself according to the space available at varying degrees of gastric fullness (Fig. 2).

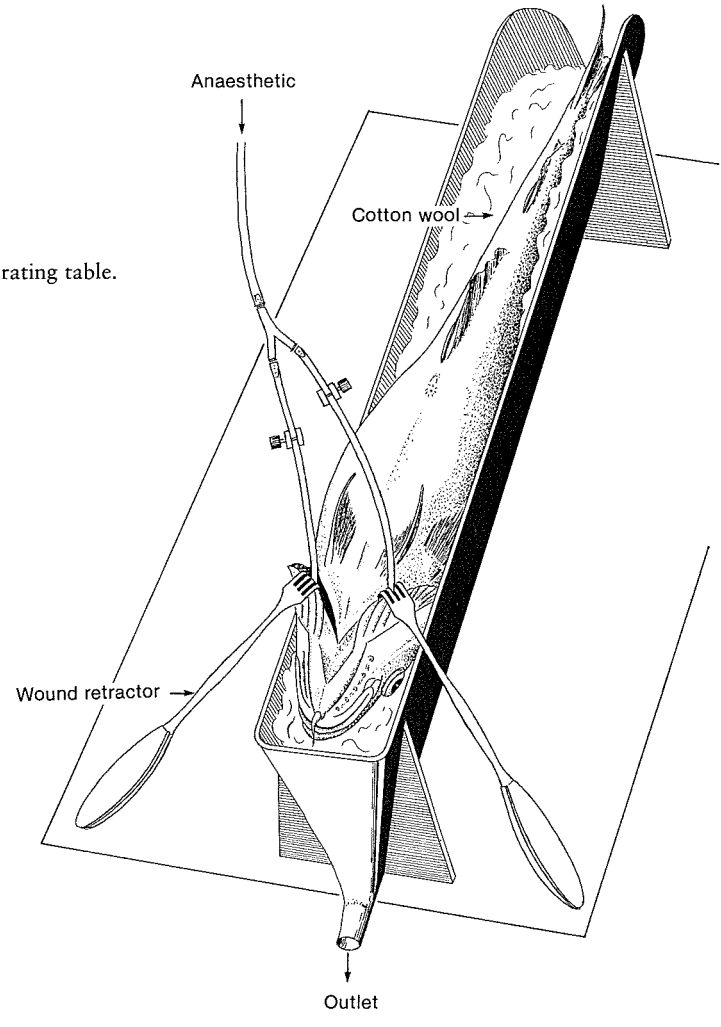

Fig. 1. Operating table.

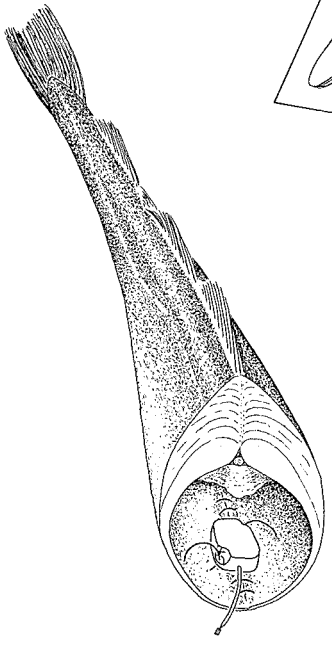

Fig. 2. Location of dummy transmitter in the body cavity of the fish.

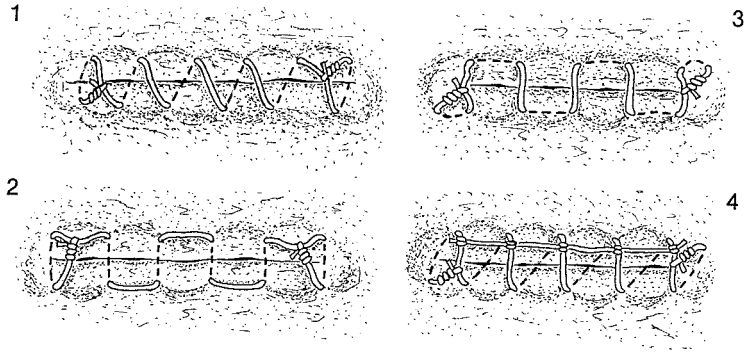

Fig. 3. Suturing techniques.

The suturing techniques employed in closing the abdominal wall varied (Fig. 3). In all cases, a continuous suture was used, and the peritoneum, abdominal musculature and the skin were sutured simultaneously. When the operation was completed, the fish was introduced into a net and immersed in salt water for 2-5 minutes. During this time its mouth was forced open and the fish pulled through the water in order to ensure a rigorous flushing of the gills. As soon as opercular and fin movements were clearly evident, the fish was transferred to a 3 m^3 tank. Immediately after this transfer the fish were capable of maintaining a normal orientation in the water and to exhibit normal muscular coordination during swimming. The fish were monitored for imflammation, behaviour and feeding every other day until healing of the wound.

Operations lasted from 15-35 minutes. During this time it was necessary either to increase or decrease the flow of the anaesthetic. The flow was increased at the time of abdominal incision and when fastening the dummy transmitter. Furthermore, the dosage was increased if muscular spasms occurred. At all other times, the flow was minimized to a slow dripping or even turned off completely in order to avoid overdosing, which may kill the fish. Whenever the anaesthetic was turned off, the gills were moistened by 0.9% NaCl. The same saline solution was applied to the skin at intervals in order to keep the integument intact.

To deter infection, all instruments were boiled for at least 30 minutes in demineralized water prior to operating. In addition, the suturing thread was sterile and the operating table and dummy transmitters had been soaked in alcohol (65%) for at least 20 minutes. Sterile surgical gloves were used by the surgeon.

Results

Prior to the series of operations described here, trials were run in order to estimate the necessary concentration of the anaesthetic. It was found that MS 222 diluted 1:20000 was sufficient to anaesthetize cod in the length interval of 35-45 cm. These fish could be anaesthetized for more than 50 minutes without observable side effects.

With the technique described in the present study 10 cod were operated. The results appear from Table 1.

Table 1. Variables expressed in days after the operation.

No.	Feeding starts	Inflammation starts	Inflammation subsides	Healing	Killing
1	3	6	13	24	84
2	3	6	11	24	84
3	2	5	12	almost healed	22
4	2	5	12	23	83
5	3	6		wound opens	21
6	3	6	10	27	39
7	3	6	10	27	130
8	2	5	8	27-34	69
9	23	7	15	27-34	69
10	1	7	15	27	69

The fish were offered food 1-3 days postoperatively and, with one exception, they started to feed immediately after food was introduced. An inflammatory response around the wound was noticed 5-7 days after the operation. It appeared as a redness of the skin which could spread a few centimeters laterally from the incision. The inflammatory response subsided in 4-8 days and the wound was completely healed 24-34 days after the operation. Only one fish (No. 5) did not heal as its wound had opened at the anterior end of the incision. This was probably due to the presence of too tight a suture. Another fish (No. 3) was almost healed when it left the experiment by jumping out of the tank. The musculature in this fish adhered well across the incision, but the skin had not had sufficient time to complete the healing process. In all other cases, the sutures were removed at healing. In case of non-absorbable sutures (TI CRON and Mersilene) the fish were anaesthetized. When absorbable sutures had been used the suture thread was absorbed externally at the time of complete healing. It should be noted, that Chrom Catgut took longer to be absorbed externally than Plain Catgut. Neither of the Catgut types were fully resorbed internally at the time of killing.

Table 2 shows the type of suture thread used for the individual fish. The sutures are stated by a number, which refers to Fig. 3. All the mentioned types of sutures

Table 2. Suturing materials and suturing techniques.

No.	Suture thread internal	Suture thread external	Suture type	Dummy implanted
1	TI1 CRON	TI1 CRON	2	+
2	–	TI1 CRON	3	–
3	–	TI1 CRON	4	–
4	–	TI1 CRON	1	–
5	–	Mersilene 2-0	2	–
6	–	Mersilene 2-0	1	–
7	Mersilene 2-0	Mersilene 2-0	2	+
8	Mersilene 2-0	Plain Catgut 2-0	1	+
9	Mersilene 2-0	Plain Catgut 2-0	1	+
10	Mersilene 2-0	Chrom Catgut 2-0	1	+

and suturing material can be used with a satisfactory result, but the type of suture marked 'I' is the easiest to make. An important finding was that thick suturing material is preferable, as pilot experiments indicated that thinner materials tend to cut through the skin and the soft musculature. A 2-0 suturing thread is well suited both for internal and external purposes. In addition, it is important that the sutures be very loose. If they are too tight they will erode the skin as well as the musculature. It is unnecessary to close the wound watertight, as the muscles around the incision contract immediately after the fish has been submerged in sea water.

At varying times after operation, fish were killed and the internal organs studied. In no case was any sign of inflammatory reaction or damage to any organs observed. A most important finding was, that dummy transmitters were encapsulated by fibrin, which made the dummy adhere to the ventral part of the abdominal wall. This is considered an advantage, as the adherence lessens the strain on the internal sutures. Another constant finding was that some of the ventral pyloric caeca adhered to the abdominal wall. Their gross morphology was indistinguishable from that of the other caeca.

With respect to the strength of the wound it was found that the scar tissue could not be torn apart. The scar tissues appeared to have the same strength and integrity as the surrounding tissues.

All fish were in excellent condition at the time of killing.

Discussion

Only few authors have used surgical implantation of transmitters. In no case were the ultrasonic transmitters or dummies fastened inside the fish, and a continuous control of the depth of anaesthesy was not attempted.

Anaesthesy:

Hart & Summerfelt (1975) have implanted ultrasonic tags into the abdominal cavity of flathead catfish (*Pylodictis olivaris*). The fish were submerged in an anaesthetic bath during operations, and the water level was adjusted so that only the abdomen protruded. Warden & Lorio (1975) implanted sonic tags into the abdomen of 16 largemouth bass (*Micropterus salmoides*). Prior to operation the fish were anaesthetized in a bath, and during the actual tagging they were transferred to a tub containing fresh water only. Henderson *et al.* (1966) inserted dummy transmitters in the abdomen of white bass (*Roccus crysops*). The fish were not anaesthetized. Ziebell (1973) implanted ultrasonic tags into the abdomen of 7 channel catfish (*Ictalurus punctatus*). During the operation, which lasted for 8 minutes, the anaesthetic was administered to the gills with a squeeze bottle once per minute.

Incisions and sutures:

Hart & Summerfelt (1975) incised in the linea alba. The opening, which was very narrow (26-30 mm), was sutured successfully with nylon sutures, but when chromic acid treated gut sutures (Catgut) were tried, the incisions failed to heal. Henderson

et al. (1966) inserted the dummy tags through a slit in the body wall just posterior to the right pelvic fin. The incision was not closed. Only 6.2 % of the operated fish were recaptured versus 18.8 % of fish having a stomach tag. In our opinion it is important to suture the wound in order to deter infection. Ziebell (1973) used a 3.5 cm incision to the left of the midventral line in front of the pelvis. The incision was closed with surgical silk 000 by a continuous suture.

Conclusion

From the techniques used it can be inferred that the implantations done by previous authors have all been of short duration.

As it is our aim to monitor physiological variables at specific locations inside the fish the need arose to fasten the transmitting unit as well as its sensor. Furthermore, in order to make space available for the process of internal suturing, the incisions had to be longer than previously described. The time spent in operating the fish was thus prolonged, the implantations lasting 35-40 minutes. This implicated that anaesthesy had to be continuous. In addition, as muscular spasms consistently occurred when perforating the peritoneum, the depth of anaesthesy had to be controlled. An accurate control of anaesthesy was achieved by simply altering the flow rate of the anaesthetic to the gills.

With respect to postoperative infections it should be noted that none of the fish showed any signs of internal infection at autopsy. Infections were effectively deterred, the precautions being that sterile surgical implements were used, and that the fish did not come into contact with unsterile water during surgery. Antibiotics were not administered. With respect to suturing materials we would recommend resorbable materials (Catgut) for external suturing. This is contrary to the results of Hart & Summerfelt (1975). Especially in field studies Catgut would offer an advantage over synthetic fibers, as remaining suturing materials tend to erode the skin slightly.

Finally it should be noted that the dummy transmitter as well as its connected sensor maintained their positions for months in the interior of the fish.

The present study shows that it is possible to perform more complicated surgery on fish than hitherto reported in the literature, and that even prolonged surgery apparently did not affect the behaviour, feeding or swimming movements of the cod.

Acknowledgement

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